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Claims 15, 16, and 18-20 have been amended to recite antibodies or mixtures of antibodies "purified from sera or cells producing them." Support for this amendment can be found through the disclosure as a whole and at page 13, lines 8-18, page 15, lines 23-25, and at page 30, line 24, through page 31, line 3 of the copy of the original specification filed with this application. (Two copies of the original specification were filed with this application. Applicants will herein refer only to the copy containing a separate title page and 41 subsequent pages.) Clearly, applicants describe and disclose antibodies to various antigens, including those recited in claims 15, 16, and 18-20, in their specification as well as the generation of antibodies from antigens. No new matter enters by this amendment.

Applicants acknowledge the withdrawal of the rejection of claims 29-31 under 35 U.S.C. § 101.

Applicants acknowledge the withdrawal of the rejection of claims 15, 16, 18-20, and 29-31 under 35 U.S.C. § 112, first paragraph.

DROP

Claims 15-16 and 18-20 stand rejected under 35 U.S.C. § 103 as allegedly being unpatentable over Barre-Sinoussi et al. (1985), abstract only. Applicants respectfully traverse this rejection.

As quoted in the M.P.E.P. at § 2128 (copy enclosed for the Examiner's convenience):

> A reference is proven to be a "printed publication" "upon satisfactory showing that such document has been disseminated or otherwise made available to the extent that persons interested and ordinarily skilled in the subject matter or art, exercising reasonable diligence, can locate it." *Citing* In re Wyer, 210 U.S.P.Q. 790 (CCPA 1981) (*quoting* I.C.E. Corp. v. Armco Steel Corp., 148 U.S.P.Q. 537, 540 (SDNY 1966)).

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Thus, it is incumbent upon the PTO to prove that a document can be "prior art" as a "printed publication" under 35 U.S.C. § 102(a) and not applicants burden to show the contrary.

In Paper No. 25, the Barre-Sinoussi et al. (1985) abstract is asserted to comply with the above definition of "printed publication" by being published prior to applicants' filing date in prior application 06/706,562, filed February 28, 1985. If that is not the case, then Barre-Sinoussi et al. cannot be "prior art" under 35 U.S.C. § 102(a) and this rejection cannot stand. Applicants will show below that, from the documentary evidence of record, there does not appear to be any reason to believe that the Barre-Sinoussi et al. (1985) abstract was actually published or appropriately available prior to February 28, 1985. Therefore, it cannot be "prior art" and this rejection should be withdrawn.

Applicants' enclose Exhibit 1 with this Amendment showing the date of the Chemical Abstracts volume containing the Barre-Sinoussi et al. (1985) abstract. The date that abstract was accessible to the public is on or about November 18, 1986, according to the date stamp on the cover page. The text of the Chemical Abstract copy of the Barre-Sinoussi et al. (1985) abstract is identical to the abstract cited by the Examiner.

Applicants have previously submitted documentary evidence showing that the copyright information for the Barre-Sinoussi et al. work states a publication date of April 9, 1986 (Exhibit 1 of the Amendment filed July 11, 1995). In addition, information in a signed letter from the publishers of the published meeting proceedings, which includes the Barre-Sinoussi et al. work, indicates that it was published April 8, 1986 (Letter filed July 24, 1995).

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There is no showing on the record of an abstract of that published work that was available before April 8, 1986. The full text work, enclosed herewith as Exhibit 2, nowhere contains the same abstract text as that cited as "prior art." Thus, it would appear that the document cited as "prior art" is the Chemical Abstract version of the Barre-Sinoussi et al. work, in which case it would not have been appropriately available until November of 1986.

The Examiner also asserts that "the data contained in the abstract was presented at the International Symposium on Retroviruses and Human Pathology, held on September 24-26, 1984." (Paper No. 25 at page 6.) However, there is no apparent evidence from the text or accompanying the text of the abstract or the pages from the published meeting proceedings to confirm this. The Examiner further alleges that "one would anticipate that copies of said abstract were available at the meeting or shortly thereafter." (Paper No. 25 at page 6.) Again, there is nothing within the four corners of the documents to confirm or even suggest this.

In effect, the Examiner's conclusions operate to shift the burden to applicants to show that the document is not "prior art." However, there is no "satisfactory showing" concerning the dissemination or availability of the Barre-Sinoussi et al. (1985) abstract as required by the M.P.E.P. and the caselaw. Because a satisfactory showing has not been made, the Barre-Sinoussi et al. (1985) abstract cannot be "prior art."

In addition, the Examiner discusses applicants' claims to priority under 35 U.S.C. § 119, at pages 6-7 of Paper No. 25, and concludes that the claim of foreign priority is moot.

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1300 I STREET, N. W.
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However, applicants submit that at least page 13, line 8, through page 15, line 25 of the instant specification discloses each of the following: p25, p18, p12, p15, p36, p42, and p80. These same proteins are similarly disclosed in applicants' foreign priority document SA 84/7005, filed September 6, 1984, at pages 8-9 (copy enclosed for the Examiner's convenience). Accordingly, at least this foreign priority document supports the disclosure and claims of this application. Foreign priority under 35 U.S.C. § 119 is, therefore, claimed and appropriate. In this case, such foreign priority antedates the earliest asserted publication date (the symposium held on September 24-26, 1984) of the Barre-Sinoussi et al. (1985) abstract.

For at least these reasons, applicants respectfully request the withdrawal of this rejection.

DROP

Claims 15, 16, and 18-20 stand rejected under 35 U.S.C. § 101 as the invention is allegedly directed towards non-statutory subject matter. Applicants respectfully traverse this rejection.

Without acquiescing to this rejection or the alleged basis for it, applicants have amended claims 15, 16, and 18-20. Amended claims 15, 16, and 18-20 now recite antibodies or mixtures of antibodies isolated from sera or cells producing them. Naturally occurring antibodies are not isolated from sera. Therefore, the claims recite subject matter sufficiently distinguished over naturally occurring antibodies. This rejection has been overcome.

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The specification is objected to and claims 29-31 rejected under 35 U.S.C. § 112, first paragraph, as the specification allegedly does not provide support for the invention as now claimed. Applicants respectfully traverse the objection and the rejection.

The Examiner asserts that "support does not exist for the term 'immunological complex' as recited in claims 29-31." (Paper No. 25 at page 9.) However, applicants disclose "immunocomplexes" and "immune complexes" at page 12, lines 5-21, and the legend of FIG. 5 at page 37 of the copy of the original specification. While literal support for claim language is not the appropriate standard for 35 U.S.C. § 112, first paragraph, (see Vas-Cath Inc. v. Mahurkar, 19 U.S.P.Q.2d 1111 (Fed. Cir. 1991)) applicants submit that the terms used in their specification are as close to the literal support for "immunological complexes," as recited in claims 29-31, as one skilled in the art would need.

In addition, the application as a whole supports the immunological complexes claimed in claims 29-31. For example, page 17, line 19, through page 18, line 10, describes assays where one skilled in the art would understand immunological complexes are clearly present and, therefore, disclosed. The results disclosed in FIG. 5 also include immunological complexes where one skilled in the art would know that the complexes necessarily are used to identify proteins in the gel (specification at page 37). One skilled in the art would certainly derive "immunological complexes" from such descriptions and understand that they were contemplated by the applicants at the time.

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1300 I STREET, N. W.
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For these reasons, applicants respectfully request withdrawal of the objection and the rejection.

DROP

Claims 16, 19, 30, and 31 stand rejected under 35 U.S.C. § 102(a) as allegedly being anticipated by McDougal et al., J. Immunol. Methods 76:171-183, 1985 (McDougal). Applicants respectfully traverse this rejection.

Claims 16, 19, 30, and 31 recite, in part, antibodies to p25, p18, and, alternatively, p12. As noted above, applicants claim priority of South African application SA 84/7005, filed September 6, 1984. At page 7, line 20, through page 8, line 23 of that application, applicants disclose antibodies and immune complexes to each of p25, p12 and p18. That disclosure supports applicants' claimed invention of claims 16, 19, 30, and 31. Since that disclosure also antedates the publication of McDougal, the McDougal document is not "prior art" to these claims.

The Examiner also alleges that antibodies to and immune complexes with proteins other than p18 and p25 are disclosed in McDougal (Paper No. 25 at page 10). Applicants do not in any way acquiesce in or agree to those statements. However, as only claims 16, 19, 30, and 31 are rejected under 35 U.S.C. § 102(a), applicants need not show the novelty of other claims in this response. Furthermore, claim 15, reciting in part an antibody to p12, is not rejected over McDougal.

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For these reasons, applicants respectfully request the withdrawal of this rejection.

DROP

Claims 15-16, 18-20, and 29-31 stand rejected under 35 U.S.C. § 103 as allegedly being unpatentable over Luciw and Dina, U.S. Patent 5,156,949 (Luciw). Applicants respectfully traverse this rejection.

The Examiner notes the alleged priority date of Luciw of October 31, 1984. However, Luciw matured from prior continuation-in-part applications. Therefore, the disclosure of the Luciw patent document does not necessarily reflect the subject matter allegedly disclosed October 31, 1984. Each prior application will have to be examined for the appropriate date specific disclosures were allegedly first made.

In addition, as noted above, the identification and isolation of antibodies to and immune complexes with the recited proteins of p12, p15, p18, p25, p36, p42, and p80 find support in applicants' foreign priority document SA 84/7005. As this document was filed prior to even the earliest claimed date of Luciw, the Luciw patent document is not "prior art" to the claims of this application.

Accordingly, applicants respectfully request the withdrawal of this rejection.

DROP

Claims 15-16, 18-20, and 29-31 stand rejected under 35 U.S.C. § 103 as allegedly being unpatentable over the combined teachings of McDougal and Barre-Sinoussi et al. (1985) abstract. Applicants respectfully traverse this rejection.

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& DUNNER, L.L.P.
1300 I STREET, N. W.
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As shown above, neither of the McDougal or Barre-Sinoussi et al. (1985) abstract documents is appropriate "prior art" to the claims of this application. Accordingly, applicants respectfully request the withdrawal of this rejection.

If there are any other fees due in connection with the filing of this Amendment, please charge such fees to our Deposit Account No. 06-0916. If an extension of time is required under 37 C.F.R. § 1.36 and not accounted for above, such an extension is respectfully requested and the fee should be charged to Deposit Account No. 06-0916.

Respectfully Submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

David J. Kulik
Registration No. 36,576

Date: February 15, 1996
Encls: 1) Exhibit 1; Chemical Abstracts of November 1986
2) Exhibit 2; copy of Barre-Sinoussi et al. in "Retroviruses and Human Pathology"
3) copy of certified South African application SA 84/7005, filed September 6, 1984
4) copy of M.P.E.P. § 2128 (1995)

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1300 I STREET, N. W.
WASHINGTON, DC 20005
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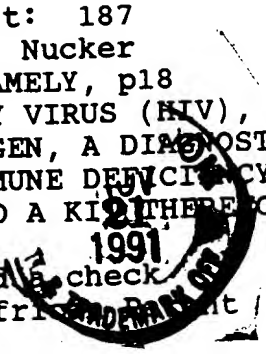
PLEASE STAMP TO ACKNOWLEDGE RECEIPT OF THE FOLLOWING:

In re Application of MONTAGNIER et al.
Serial No.: 07/158,073 Group Art Unit: 187
Filed: February 12, 1988 Examiner: C. Nucker
For: ANTIGEN OF A HUMAN RETROVIRUS, NAMELY, p18 PROTEIN OF HUMAN IMMUNODEFICIENCY VIRUS (HIV), COMPOSITIONS CONTAINING THE ANTIGEN, A DIAGNOSTIC METHOD FOR DETECTING ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS) AND PRE-AIDS, AND A KIT THEREFOR



Petition Under 37 C.F.R. §1.55 and a check in the amount of $120.00; South Afri[illegible]nt Application No. 84/7005

Dated: November 21, 1991
Attorney Docket No. 03495.0004-02000
KJM/MMSchafer/leb

11/21/91

PATENT
ATTORNEY DOCKET NO. 03495.0004-02000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
	)	
MONTAGNIER et al.	)	Batch No. R83
	)	
Serial No.: 07/158,073	)	Group Art Unit: 187
	)	
Filed: February 12, 1988	)	Examiner: C. Nucker
	)	

For: ANTIGEN OF A HUMAN RETROVIRUS, NAMELY, p18 PROTEIN OF HUMAN IMMUNODEFICIENCY VIRUS (HIV), COMPOSITIONS CONTAINING THE ANTIGEN, A DIAGNOSTIC METHOD FOR DETECTING ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS) AND PRE-AIDS, AND A KIT THEREFOR

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

PETITION UNDER 37 C.F.R. §1.55

In accordance with 37 C.F.R. §1.55, applicants request the entry of priority document South Africa No. 84/7005, filed herewith. Applicants also file herewith the fee for entry of late priority papers of $120.00, as set forth in 37 C.F.R. §1.17(i)(1).

Applicants acknowledge that this priority document is being submitted after payment of the issue fee. Due to the protracted prosecution history of this application, it was discovered late that although the claim for priority had been made, the priority document had inadvertently not been filed. The request for a certified copy of the priority document was further complicated

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& DUNNER
00 I STREET, N. W.
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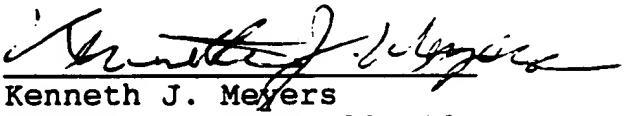
by the fact that the assignee is represented by French counsel, while the priority document is South African. The undersigned received the priority document one day after payment of the issue fee.

Applicants note that it was not necessary to rely on the priority date of South Africa No. 84/7005 during prosecution of this application. This submission is merely formal and does not result in prejudice to another party.

If there are any other fees due in connection with the filing of this Petition, please charge such fees to our Deposit Account No. 06-916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER

By: 
Kenneth J. Meyers
Registration No. 25,146

Dated: November 21, 1991

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FARABOW, GARRETT
& DUNNER
1300 I STREET, N. W.
WASHINGTON, DC 20005
1-202 408-4000

Sertifikaat

PATENTKANTOOR

DEPARTEMENT VAN HANDEL EN NYWERHEID

REPUBLIEK VAN SUID-AFRIKA

REPUBLIC OF SOUTH AFRICA

Certificate

PATENT OFFICE

DEPARTMENT OF TRADE AND INDUSTRY

Hiermee word gesertifiseer dat
This is to certify that

the attached documents are true and correct copies of the letters patent document, form P2, complete specification and claims in respect of
SOUTH AFRICAN PATENT 84/7005
in the name of
INSTITUT PASTEUR, Etablissement Public
for an invention entitled
ANTIGENS, MEANS AND METHOD FOR THE DIAGNOSIS OF LYMPHADENOPATHY AND ACQUIRED IMMUNE DEFICIENCY SYNDROME
Filed: 6 September 1984
Accepted: 6 September 1985
Advertised and Granted: 27 November 1985
Priority claimed: GB - 8324800 - 15.9.83

Getek... Signed ...bliek van Suid-Afrika, hierdie ...c of South Africa, this 30th dag van day of October 1991

Registrateur van Patente
Registrar of Patents

H 573

DEPARTMENT OF TRADE AND INDUSTRY
DEPARTEMENT VAN HANDEL EN NYWERHEID

REPUBLIC OF SOUTH AFRICA

REPUBLIEK VAN SUID-AFRIKA

LETTERS PATENT

(PATENTS ACT, 1978)

PATENTBRIEF

(WET OP PATENTE, 1978)

No. 84/7005

WHEREAS INSTITUT PASTEUR, Etablissement Public
NADEMAAL (Hereinafter called "the Patentee")
(Hieronder "die Patenthouer" genoem)

has applied to me for the grant of a patent in respect of an invention described and claimed in the complete specification deposited at the aansoek by my gedoen het om die verlening van 'n patent ten opsigte van 'n uitvinding wat beskryf is en waarop aanspraak gemaak word

Patent Office under the above-mentioned number, a copy of which is annexed, together with the relevant Form P. 2;
in die volledige spesifikasie wat by die Patentkantoor onder bovermelde nommer ingedien is en waarvan 'n afskrif aangeheg is tesame met

die betrokke vorm P. 2;

NOW THEREFORE these Letters Patent are to grant to the Patentee a patent, the effect of which shall be to grant to the Patentee
SO IS DIT dat hierdie Patentbrief aan die Patenthouer 'n patent verleen wat die uitwerking het dat, behoudens die bepalings van die
in the Republic, subject to the provisions of the Act, for the duration of the patent, the right to exclude other persons from making, using,
Wet, aan die Patenthouer vir die duur van die patent in die Republiek die reg verleen word om ander persone uit te sluit van die vervaar-

exercising or disposing of the invention, so that he shall have and enjoy the whole profit and advantage accruing by reason of the invention.
diging, aanwending, uitoefening of van die handsetting van die uitvinding, sodat hy al die wins en voordeel wat uit die uitvinding voort-

spruit, verkry en geniet.

IN TESTIMONY WHEREOF the seal of the Patent Office has been affixed at Pretoria with effect from the
TER BETUIGING WAARVAN die seël van die Patentkantoor hierop te Pretoria aangebring is met ingang van die

27TH day of NOVEMBER nineteen hundred and EIGHTY-FIVE
dag van eenduisend negehonderd

Registrar of Patents/Registrateur van

FORM P.2

REPUBLIC OF SOUTH AFRICA | REGISTER OF PATENTS | PATENTS ACT, 1978

OFFICIAL APPLICATION NO.	LODGING DATE: PROVISIONAL	ACCEPTANCE DATE
21 01 847005	22	47 6-9-85
INTERNATIONAL CLASSIFICATION	**LODGING DATE: COMPLETE**	**GRANTED DATE**
51 A61B, A61K	23 84.09.06	1985-11-27

FULL NAME(S) OF APPLICANT(S)/PATENTEE(S)

71 INSTITUT PASTEUR, Etablissement Public

APPLICANTS SUBSTITUTED:	DATE REGISTERED
71	

ASSIGNEE(S)	DATE REGISTERED
71	

FULL NAME(S) OF INVENTOR(S)

72

F. BARRE-SINOUSSI; J.C. CHERMANN; F. REY; M.T. NUGEYRE; S. CHAMARET; J. GRUEST; C. DAUGUET; C. AXLER-BLIN; F. VEZINET-BRUN; C. ROUZIOUX; W. ROZENBAUM and L. MONTAGNIER.

PRIORITY CLAIMED	COUNTRY	NUMBER	DATE
N.B. Use International abbreviation for country (See Schedule 4)	33 GB	31 8324800	32 83.09.15

TITLE OF INVENTION

54 ANTIGENS, MEANS AND METHOD FOR THE DIAGNOSIS OF LYMPHADENOPATHY AND ACQUIRED IMMUNE DEFICIENCY SYNDROME

ADDRESS OF APPLICANT(S)/PATENTEE(S)

28, rue du Dr. Roux, 75015 PARIS, France

ADDRESS FOR SERVICE	S & F REF
74 SPOOR & FISHER, PRETORIA	41558/ch

PATENT OF ADDITION NO.	DATE OF ANY CHANGE	

SPOOR AND FISHER

FORM P.1.

REVENUE STAMPS OR REVENUE FRANKING MACHINE IMPRESSION

OFFICIAL DATE STAMP

REPUBLIC OF SOUTH AFRICA
PATENTS ACT, 1978

APPLICATION FOR A PATENT

AND ACKNOWLEDGEMENT F RECEIPT
(Section 30 (1) – Regulation 22)

The grant of a patent is hereby requested by the undermentioned applicant on the basis of the present application filed in duplicate

OFFICIAL APPLICATION No.

21	01	847005

S & F REFERENCE

41558/th

FULL NAME(S) OF APPLICANT(S)

71 INSTITUT PASTEUR, Etablissement Public

ADDRESS(ES) OF APPLICANT(S)

28, rue du Dr. Roux, 75015 PARIS, France

TITLE OF INVENTION

54 ANTIGENS, MEANS AND METHOD FOR THE DIAGNOSIS OF LYMPHADENOPATHY AND ACQUIRED IMMUNE DEFICIENCY SYNDROME

PRIORITY IS CLAIMED AS SET OUT ON THE ACCOMPANYING FORM P.2.

THIS APPLICATION IS FOR A PATENT OF ADDITION TO PATENT APPLICATION NO.

21	01	

THIS APPLICATION IS A FRESH APPLICATION IN TERMS OF SECTION 37 AND BASED ON APPLICATION NO.

21	01	

THIS APPLICATION IS ACCOMPANIED BY:

- [X] 1. ~~A single copy of a provisional or~~ two copies of a complete specification of 27 pages.
- [ ] 2. Drawings of ..... sheets
- [X] 3. Publication particulars and abstract (Form P.8. in duplicate)
- [ ] 4. A copy of Figure ..... of the drawings (if any) for the abstract
- [ ] 5. Assignment of invention
- [ ] 6. Certified priority document(s) (State number)
- [ ] 7. Translation of the priority document(s)
- [ ] 8. An assignment of priority rights
- [ ] 9. A copy of the Form P.2. and the specification of S.A. Patent Application No | 21 | 01 | |
- [ ] 10. A declaration and power of attorney on Form P.3.
- [ ] 11. Request for ante-dating on Form P.4.
- [ ] 12. Request for classification on Form P.9.
- [ ] 13.

74 ADDRESS FOR SERVICE: SPOOR AND FISHER, SANDTON, PRETORIA, ~~DURBAN~~

REGISTRAR OF PATENTS, TRADE MARKS, DESIGNS, COPYRIGHT
RECEIVED
OFFICIAL DATE STAMP
06 -09- 1984
PRETORIA

Dated this 6 Day of September 1984

Z/10

SPOOR AND FISHER

FORM P.7.
(To be lodged in duplicate)

REPUBLIC OF SOUTH AFRICA
PATENTS ACT, 1978

COMPLETE SPECIFICATION

(Section 30(1) – Regulation 28)

OFFICIAL APPLICATION NO.		LODGING DATE	
21 01	847005	22	84.09.06

INTERNATIONAL CLASSIFICATION

51	A61B A61K

FULL NAME(S) OF APPLICANT(S)

71	INSTITUT PASTEUR, Etablissement Public

FULL NAME(S) OF INVENTOR(S)

72	F. BARRE-SINOUSSI; J.C. CHERMANN; F. REY; M.T. NUGEYRE; S. CHAMARET; J. GRUEST; C. DAUGUET; C. AXLER-BLIN; F. VEZINET-BRUN; C. ROUZIOUX; W. ROZENBAUM and L. MONTAGNIER.

TITLE OF INVENTION

54	ANTIGENS, MEANS AND METHOD FOR THE DIAGNOSIS OF LYMPHADENOPATHY AND ACQUIRED IMMUNE DEFICIENCY SYNDROME

The invention relates to antigens, means and methods for the diagnosis of lymphadenopathy and acquired immune deficiency syndrome.

The acquired immune deficiency syndrome (AIDS) has recently been recognized in several countries. The disease has been reported mainly in homosexual males with multiple partners, and epidemiological studies suggest horizontal transmission by sexual routes as well as by intravenous drug administration, and blood transfusion. The pronounced depression of cellular immunity that occurs in patients with AIDS and the quantitative modifications of subpopulations of their T lymphocytes suggest that T cells or a subset of T cells might be a preferential target for the putative infectious agent. Alternatively, these modifications may result from subsequent infections. The depressed cellular immunity may result in serious opportunistic infections in AIDS patients, many of whom develop Kaposi's sarcoma. However, a picture of persistent multiple lymphadenopathies has also been described in homosexual males and infants who may or may not develop AIDS. The histological aspect of such lymph nodes is that of reactive hyperplasia. Such cases may correspond to an early or a milder form of the disease.

It has been found that one of the major etiological agents of AIDS and of lymphadenopathy syndrom (LAS), which is often considered as a prodromic sign of AIDS, should consist of a T-lymphotropic retrovirus which has been isolated from a lymph node of a homosexual patient with multiple lymphadenopathies. The virus appears to be distinct from the human

T-cell leukemia virus (HTLV) family (R.C. Gallo and M. S. Reitz, "J. Natl. Cancer Inst.", 69 (No. 6), 1209 (1982)). The last mentioned virus has been known as belonging to the so-called HTLV-I subgroup.

The patient was a 33-year-old homosexual male who sought medical consultation in December 1982 for cervical lymphadenopathy and asthenia (patient 1). Examination showed axillary and inguinal lymphadenopathies. Neither fever nor recent loss of weight were noted. The patient had a history of several episodes of gonorrhea and had been treated for syphilis in September 1932. During interviews he indicated that he had had more than 50 sexual partners per year and had travelled to many countries, including North Africa, Greece, and India. His last trip to New York was in 1979.

Laboratory tests indicated positive serology (immunoglobulin G) for cytomegalovirus (CMV) and Epstein-Barr virus. Herpes simplex virus was detected in cells from his throat that were cultured on human and monkey cells. A biopsy of a cervical lymph node was performed. One sample served for histological examination, which revealed follicular hyperplasia without change of the general structure of the lymph node. Immunohistological studies revealed, in paracortical areas, numerous T lymphocytes ($OKT3^+$). Typing of the whole cellular suspension indicated that 68 percent of the cells were T lymphocytes ($OKT3^+$), 44 percent were T-helper cells ($OKT4^+$), and 16 percent were suppressor cells ($OKT8^+$).

Cells of the same biopsed lymph node were put in culture medium with phytohemagglutinin (PHA), T-cell growth factor (TCGF), and antiserum to human α interferon ("The cells were grown in RPMI-1640 medium supplemented with antibiotics, 10^{-5}M β-mercaptoétnanol, 10 percent fetal calf serum, 0.1 percent sheep antibody to human α interferon (neutralizing titer, 7 IU at 10^{-5}dilution and 10 percent TCGF, free of PHA"). The reason for using the antiserum

to α-interferon was to neutralize endogenous interferon which is secreted by cells chronically infected by viruses, including retroviruses. In the mouse system, it had previously been shown that anti-serum to interferon could increase retrovirus production by a factor of 10 to 50 (F. Barré-Sinoussi et al., "Ann. Microbiol. (Institut Pasteur)" 130B, 349 (1979). After 3 days, the culture was continued in the same medium without PHA. Samples were regularly taken for reverse transcriptase assay and for examination in the electron microscope.

After 15 days of culture, a reverse transcriptase activity was detected in the culture supernatant by using the ionic conditions described for HTLV-I (B.J. Poiesz et al. "Proc. Natl. Acad. Sci. U.S.A." 77, 7415 (1980)). Virus production continued for 15 days and decreased thereafter, in parallel with the decline of lymphocyte proliferation. Peripheral blood lymphocytes cultured on the same way were consistently negative for reverse transcriptase activity, even after 6 weeks. Cytomegalovirus could be detected, upon prolonged co-cultivation with MRC5 cells, in the original biopsy tissue, but not in the cultured T lymphocytes at any time of the culture.

The invention relates to the newly isolated virus as a source of the above said antigen which will be defined later.

The newly isolated virus, which will hereafter be termed as LAV_1, will however be described first.

The virus is transmissible to cultures of T lymphocytes obtained from healthy donors. Particularly virus transmission was attempted with the use of a culture of T lymphocytes established from an adult healthy donor of the Blood Transfusion Center at the Pasteur Institute. On day 3, half of the culture was co-

cultivated with lymphocytes from the biopsy after centrifugation of the mixed cell suspensions. Reverse transcriptase activity could be detected in the supernatant on day 15 of the coculture but was not detectable on days 5 and 10. The reverse transcriptase had the same characteristics as that released by the patient's cells and the amount released remained stable for 15 to 20 days. Cells of the uninfected culture of the donor lymphocytes did not release reverse transcriptase activity during this period or up to 6 weeks when the culture was discontinued.

The cell-free supernatant of the infected coculture was used to infect 3-day-old cultures of T lymphocytes from two umbilical cords, LC1 and LC5, in the presence of Polybrene (2 µg/ml). After a lag period of 7 days, a relatively high titer of reverse transcriptase activity was detected in the supernatant of both cord lymphocyte cultures. Identical cultures, which had not been infected, remained negative. These two successive infections clearly show that the virus could be propagated on normal lymphocytes from either new-borns or adults.

In the above co-cultures one used either the cells of patient 1 as such (they declined and no longer grew) or cells which had been pre-X-rayed or mitomycin C-treated.

The LAV_1 virus, or LAV_1 virus suspensions, which can be obtained from infected cultures of lymphocytes have characteristics which distinguish them completly from other HTLV. These characteristics will be referred to hereafter and, when appropriate, in relation to the drawing. It shows curves representative of variation of reverse transcriptase activity and [^{3}H]uridine activity respectively versus successive fractions of the LAV_1 virus in the sucrose gradient, after ultracentrifugation therein of the virus contents of a cell-free supernatant obtained from a culture of infected lymphocytes.

The analysis of LAV_1 virus by resorting to reverse transcriptase activity can be carried out according to the procedure which was used in relation to virus from patient 1, on fig. 1. The results of the analysis are illustrated on fig. 1. Cord blood T lymphocytes infected with virus from patient 1 were labelled for 18 hours with [^{3}H]uridine (28 Ci/mmole, Amersham ; 20 µCi/ml). Cell-free supernatant was ultracentrifuged for 1 hour at 50,000 rev/min. The pellet was resuspended in 200 µl of NTE buffer (10 mM tris, pH 7.4, 100 mM NaCl, and 1 mM EDTA) and was centrifuged over a 3-ml linear sucrose gradient (10 to 60 percent) at 55,000 rev/min for 90 minutes in an IEC type SB 493 rotor . Fractions (200 µl) were collected, and 30 µl samples of each fraction were assayed for DNA RNA dependant polymerase activity with 5 mM Mg^{2+} and poly(A)-oligo-$(dT)_{12-18}$ as template primer ; a 20-µl portion of each fraction was precipitated with 10 percent trichloroacetic acid and then filtered on a 0.45-µm Millipore filter. The ^{3}H-labelled acid precipitable material was measured in a Packard 8 counter.

That the new virus isolate was a retrovirus was further indicated by its density in the above sucrose gradient, which was 1.16, and by its labelling with [^{3}H]uridine (fig. 1). A fast sedimenting RNA appears to be associated with the LAV_1 virus.

Virus-infected cells from the original biopsy as well as infected lymphocytes from the first and second viral passages were used to determine the optimal requirements for reverse transcriptase activity and the template specificity of the enzyme. The results were the same in all instances. The reverse transcriptase activity displayed a strong affinity for poly(adenylate-oligodeoxythymidylate)[poly(A)-oligo$(dT)_{12-18}$], and required Mg^{2+} with

an optimal concentration (5mM) and an optimal pH of 7.8. The reaction was not inhibited by actinomycin D. This character, as well as the preferential specificity for riboseadenylate-deoxythymidylate over deoxyadenylate-deoxythymidylate, distinguish the viral enzyme from DNA-dependent polymerases.

Electron microscopy of ultrathin sections of virus-producing cells shows two types of particles, presumably corresponding to the immature and mature forms of the virus : immature particles are budding at the cell surface, with a dense crescent in close contact with the plasma membrane. Occasionally, some particles remain in this state, while being freed from the cell surface.

Mature particles have a quite different morphology with a small, dense, eccentric core (mean diameter: 41 nM). Most of virions are round (mean diameter : 139 nM) or ovoid, but in some pictures, especially in the particles seen in the original culture from which the virus was isolated, a tailed morphology can also be observed. The latter form can also be observed in cytoplasmic vesicles which were released in the medium. Such particles are also formed by budding from vesicle membranes.

Morphology of mature particles is clearly distinct from HTLV, whose large core has a mean diameter of 92 nM.

Helper T-lymphocytes (Leu 3 cells) form the main target of the virus. In other words the LAV, virus has particular tropism for these cells. Leu 3 cells are recognizable by the monoclonal antibodies commercialized by ORTHO under the trademark OKT4. In contrast enriched cultures of Leu 2 cells, which are mainly suppressor or cytotoxic cells and which are recognized by the monoclonal antibodies commercialized by ORTHO under the

trademark OKT8 did not produce, when infected under the same conditions, any detectable RT activity even 6 weeks after virus infection.

In most cases of AIDS, the ratio of $OKT4^+$ over $OKT8^+$ cells which is normally over 1, is depressed to values as low of 0.1 or less.

The LAV_1 virus is also immunologically distinct from previously known HTLV-1 isolates from cultured T lymphocytes of patients with T lymphomas and T leukemias. The antibodies used were specific for the p19 and p24 core proteins of HTLV-1. A monoclonal antibody to p19 (M. Robert-Guroff et al. "J. Exp. Med." 154, 1957 (1981)) and a polyclonal goat antibody to p24 (V.S. Kalyanaraman et al. "J. Virol.", 38, 906 (1981)) were used in an indirect fluorescence assay against infected cells from the biopsy of patient 1 and lymphocytes obtained from a healthy donor and infected with the same virus. The LAV_1 virus-producing cells did not react with either type of antibody, whereas two lines of cord lymphocytes chronically infected with HTVL I (M. Popovic, P.S. Sarin, M. Robert-Guroff, V. S. Kalyanaraman, D. Mann, J. Minowada, R.C. Gallo, "Science" 219, 856 (1983)) and used as controls showed strong surface fluorescence.

In order to determine which viral antigen was recognized by antibodies present in the patient's sera, several immunoprecipitation experiments were carried out. Cord lymphocytes infected with virus from patient 1 and uninfected controls were labelled with [^{35}S]methionine for 20 hours. Cells were lysed with detergents, and a cytoplasmic S10 extract was made. Labelled virus released in the supernatant was banded in a sucrose gradient. Both materials were immunoprecipitated by antiserum to HTVL-1 p24, by serum from patient 1, and by serum samples from healthy donors. Immunocomplexes were

analyzed by polyacrylamide gel electrophoresis under denaturing conditions. A p25 protein present in the virus-infected cells from patient 1 and in LC1 cells infected with this virus, was specifically recognized by serum from patient 1 but not by antiserum to HTLV-1 p24 obtained under similar conditions or serum of normal donors. Conversely the p24 present in control HTLV-infected cell extracts was recognized by antibodies to HTLV but not by serum from patient 1.

The main protein (p25) detected after purification of ^{35}S-methionine-labelled virus has a molecular weight of about 25,000 (or 25K). This protein is recognized by the serum of patient 1. By analogy with other retroviruses, this major protein was considered to be located in the viral core.

This can be confirmed in immuno-electron microscopy experiments, which show that the patient's serum can agglutinate the viral cores. Conversely, an antiserum raised in rabbit against an ether treated virus did not precipitate the p25 protein.

Other proteins besides the p25 protein could be detected upon gel electrophoresis under similar denaturing conditions when the virus had previously been metabolically labelled with ^{35}S-Cysteine. These proteins have molecular weights of approximately 18,000 (p18) and 12,000 (p12). The same protein bands are also apparent after silver staining. These proteins are also recognized by the sera of patients afflicted with LAS or AIDS.

The viral origin of other proteins seen in polyacrylamide gel electrophoresis of purified virus is more difficult to assess. A p15 protein could be seen after silver staining, but was much weaker after ^{35}S-methionine perhaps due to the paucity of this amino-acid in the protein. In the higher MW range, a contamination of the virus by cellular proteins, either inside or outside the viral envelope, is likely. A 36K and a 42K protein and a 80K protein were constantly formed to be associated with the purified virus and may represent the major envelope proteins.

No p19 (or having a molecular weight of about 19 mM) was isolated from extracts of methionine-labelled LAV_1.

The invention concerns more particularly the extracts of said virus as soon as they can be recognized immunologically by sera of patients afflicted with LAS or AIDS. Needless say any type of immunological assay may be brought into play. By way of example innunofluorescence or immunoenzymatic assays or radio-immunoprecipitation tests are particularly suitable.

As a matter of fact and except under exceptional circumstances, sera of diseased patients do not recognize the intact LAV_1 virus, or viruses having similar pheno-typical or immunological properties. The envelope proteins of the virus appeared as not detectable immunologically by the patients' sera. However as soon as the core proteins become exposed to said sera, the immunological detection becomes possible. Therefore the invention concerns all extracts of the virus, whether it be the crudest ones - particularly mere virus lyzates - or the more purified ones, particularly extracts enriched in the p25 protein or even the purified p25 protein or in protein immunologically related therewith. Other preferred purified extracts obtained from the virus contain either the p12 or the p18 protein. Preferred enriched extracts comprise the 3 proteins, i.e. the p12, p18 and p25 proteins. Any purification procedure may be resorted to. By way of example only, one may use purification procedures such as disclosed by R.C. Montelaro et al, J. of virology, June 1982, pp; 1029-1038.

The invention concerns more generally extracts of any virus having similar phenotype and immunologically related to that obtained from LAV_1. Sources of viruses of the LAV type consist of T-lymphocyte cultures isolatable from LAS- and AIDS - patients or from haemophiliacs.

In that respect other preferred extracts are those obtained from two retroviruses obtained by propagation on normal lymphocytes of the retroviruses isolated from :

1) lymph node lymphocytes of a caucasian homosexual with multiple partners, having extensive Kaposi sarcoma lesions and severe lymphopenia with practically no $OKT4^+$ lymphocytes in his blood ;

2) blood lymphocytes of a young B haemophiliac presenting neurotoxoplasmosis and $OKT4^+/OKT8^+$ ratio of 0.1.

These two retroviruses have been named IDAV1 and IDAV2 respectively (for Immune Deficiency Associated Virus). Results of partial characterization obtained so far indicate similarity - if not identity - of IDAV1 and IDAV2 to LAV1 :

- same ionic requirements and template specificities of reverse transcriptase,
- same morphology in ultrathin sections,
- antigenically related p25 proteins : serum of LAV1 patient immunoprecipitates p25 from IDAV1 and IDAV2 ; conversely, serum from IDAV2 patient immunoprecipitates LAV1 p25.

IDAV1 patient serum seemed to have a lower antibodies titer and gave a weak precipitation band for LAV1 and IDAV1 p25 proteins. The p25 protein of IDAV1 and IDAV2 was not recognized by HTLV $p24$ antiserum.

These similarities suggest that all these three isolates belong to the same group of viruses.

The invention further relates to a method of in vitro diagnosis of LAS or AIDS, which comprises contacting a serum or other biological medium from a patient to be diagnosed ----------

with a virus extract as above defined or any of the preferred purified extracts referred to hereabove and detecting the immunological reaction.

Preferred methods bring into play immunoenzymatic or immunofluorescent assays, particularly according to the ELISA technique. Assays may be either direct or indirect immunoenzymatic or immunofluorescent assays.

Thus the invention also relates to labelled virus extracts whatever the type of labelling: enzymatic, fluorescent, radioactive, etc.

Such assays include for instance :

- depositing determined amounts of the extract according to the invention in the wells of titration microplate;
- introducing in said wells increasing dilutions of the serum to be diagnosed;
- incubating the microplate;
- washing the microplate extensively;
- introducing in the wells of the microplate labelled antibodies directed against blood immunoglobulines, the labelling being by an enzyme selected among those which are capable of hydrolysing a substrate, whereby the latter then undergoes a modification of its absorption of radiations, at least in a determined wavelength band and
- detecting, preferably in a comparative manner with respect to a control, the amount of substrate hydrolysis as a measure of the potential risks or effective presence of the disease.

The invention also relates to kits for the above-said diagnosis which comprise :

- an extract or more purified fraction of the abovesaid types of viruses, said extract or fraction being labelled such as by a radioactive, enzymatic or immunofluorescent label ;

- human anti-immunoglobulins or protein A (advantageously fixed on a water-insoluble support such as agarose beads) ;
- a lymphocyte extract obtained from a healty person ;
- buffers and, if appropriate, substrates for the vizualization of the label.

Other features of the invention will further appear as the description proceeds of preferred isolation and culturing procedures of the relevant virus, of preferred extraction methods of an extract suitable as diagnostic means, of a preferred diagnosis technique and of the results that can be achieved.

1. VIRUS PROPAGATION :

Cultured T-lymphocytes from either ombilical cord or blood, or also bone marrow cells from healthy, virus negative, adult donors are suitable for virus propagation.

There is however some variation from individual to individual in the capacity of lymphocytes to grow the virus. Therefore, it is preferable t select an adult healthy donor, which had no antibodies against the virus and whose lymphocytes repeatly did not release spontaneously virus, as detected by reverse transcriptase activity (RT) nor expressed viral proteins.

Lymphocytes of the donor were obtained and separated by cytopheresis and stored frozen at -130°C in liquid nitrogen, in RPMI 1640 medium, supplemented with 50 % decomplemented human serum and 10 % DMSO until used.

For viral infection, lymphocytes were put in culture (RPMI 1640 medium) with phytohaemagglutinin (PHA) at the concentration of 5.10^6 cells/ml for 3 days.

Then, the medium was removed and cells resuspended in viral suspension (crude supernatant of virus-

producing lymphocytes, stored at -80°C). Optimal conditions of cell/virus concentrations were 2.10^6 cells for 5,000 to 10,000 cpm of RT activity, the latter determined as previously described. After 24 hours, cells were centrifuged to remove the unadsorbed virus and resuspended in culture PHA-free medium and supplemented with PHA-free TCGF (Interleukin 2) : (0.5 - 1 U/ml, final concentration), POLYBREN (Sigma) 2 µg/ml and anti-interferon α sheep serum, inactivated at 56°C for 30 minutes (0.1 % of a serum which is able to neutralize 7 U of α leucocyte interferon at a 1/100,000 dilution).

Virus production was tested every 3 days by RT activity determination on 1 ml samples.

The presence of anti-interferon serum is important in virus production : when lymphocytes were infected in the absence of anti-human-α-interferon serum, virus production, as assayed by RT activity, was very low or delayed. Since the sheep antiserum used was raised against partly purified α leucocyte interferon, made according to the Cantell technique, the role of components other than interferon cannot be excluded.

Virus production starts usually from day 9 to 15 after infection, and lasts for 10-15 days. In no cases, the emergence of a continuous permanent line was observed.

2. <u>VIRUS PURIFICATION</u> :

For its use in ELISA, the virus was concentrated by 10 % Polyethyleneglycol (PEG 6000) precipitation and banded twice to equilibrium in a 20-60 % sucrose gradient. The viral band at density 1.16 is then recovered and usable as such for ELISA assays.

For use in RIPA radio-immune precipitation assay, purification in isotonic gradients of Metrizamide (sold under the trademark NYCODENZ by Nyegaard, Oslo) were found to be preferable. Viral density in such gradients was very low (1.10-1.11).

Metabolic labelling with ^{35}S-methionine of cells and virus (RIPA) followed by polyacrylamide gel electrophoresis were performed as above described, except the following modifications for RIPA : virus purified in NYCODENZ was lysed in 4 volumes of RIPA containing 500 U/ml of aprotinin. Incubation with 5 µl of serum to be tested was made for 1 hour at 37°C and then 18 hours at +4°C. Further incubation of the immunocomplexes with protein A SEPHAROSE beads was for 3 hours at +4°C.

3. PREPARATION OF THE VIRUS EXTRACT FOR ELISA ASSAYS :

Virus purified in sucrose gradient as above described, is lysed in RIPA buffer (0.5 % SDS) and coated on wells of microtest plates (Nunc).

Preferred conditions for the ELISA assay are summed up hereafter.

After addition to duplicate wells of serial dilutions of each serum to be tested, the specifically fixed IgGs are revealed by goat anti-human IgG coupled with peroxydase. The enzymatic reaction is carried out on ortho-phenylene-diamine as substrate and read with an automatic spectrophotometer at 492 nM.

On the same plate each serum is tested on a control antigen (a crude cytoplasmic lysate of uninfected T-lymphocytes from the same donor) is used

in order to eliminate unspecific binding, which can be high with some sera.

Sera are considered as positive (antibodies against the virus) when the difference between O.D. against the viral antigen and O.D. against control cellular antigen was at least 0.30.

Hereafter there is disclosed a specific test for assaying the above mentionned disease or exposure to disease risks.

Method.

This ELISA test is for detecting and titration of seric anti-retrovirus type LAV antibodies.

It comprises carrying out a competition test between a viral antigen (cultivated on T lymphocytes) and a control antigen constituted by a lysate of the same though non-infected lymphocytes.

The binding of the antibodies on the two antigens is revealed by the use of a human antiglobulin labelled with an enzyme which itself is revealed by the addition of a corresponding substrate.

Preparation of the viral antigen.

The cellular cultures which are used are T lymphocytes of human origin which come from :

. umbilical cord blood,

. bone marrow,

. blood of a healthy donnor.

After infection of the cells by the virus, the supernatant of the infected cell culture is used. It is concentrated by precipitating with 10 % PEG, then purified (two or three times) on a (20-60 %) sucrose gradient by ultracentrifugation to equilibrium.

The viral fractions are gathered and concentrated by centrifugation at 50 000 rotations per minute for 60 minutes.

The sedimented virus is taken in a minimum volume of buffer NTE at pH 7.4 (Tris 0.01 M, NaCl 0.1 M, EDTA 0.001 M).

The proteic concentration is determined by the Lowry method.

The virus is then lysed by a (RIPA + SDS) buffer (0.5 % final) for 15 minutes at 37°C.

Preparation of the control antigen.

The non-infected lymphocytes are cultured according to the preceeding conditions for from 5 to 10 days. They are centrifuged at low speed and lysed in the RIPA buffer in the presence of 5 % of the product commercialized under the name of ZYMOFREN (Spécia) (500 u/ml). After a stay of 15 minutes at 4°C with frequent stirrings with vortex, the lysate is centrifuged at 10 000 rotations per minute. The supernatant constitutes the control antigen. Its concentration in protein is measured by the Lowry method.

Reagents.

1 - Plates = NUNC - special controlled ELISA
2 - Buffer PBS : pH 7.5
3 - TWEEN 20
4 - Carbonate buffer : pH = 9.6 (CO_3Na_2 = 0.2 M
(CO_3HN_a = 0.2 M
5 - Non foetal calf serum : which is stored in frozen state (BIOPRO),
6 - Bovine serum albumine (BSA) SIGMA (fraction V)
7 - Human anti IgG (H+L) labelled with peroxydase PASTEUR, in tubes of 1 ml preserved at 4°C
8 - Washing buffer = PBS buffer, pH 7.5 + 0.05 % TWEEN 20

Dilution of the conjuguate is carried out at the dilution indicated in PBS buffer + TWEEN 20 (0.05 %) - bovine albumine 0.5 g per 100 ml

9 - Dilution buffer of sera = PBS buffer + 0.05 % TWEEN 20
+ 0.5 g BSA
bovine serum albumine per
100 ml

10 - Substrat = OPD

. Citrate buffer pH = 5.6 trisodic citrate
($C_6H_5Na_4O_3$, $2H_2O$), 0.05 M ; citric acid
($C_6H_3O_7$, $1H_2O$), 0.05 M.

. Hydrogen peroxyde = at 30 % (110 volumes) - used at 0.03 % when using citrate buffer.

. Orthophenylene diamine = SIGMA
75 mg per 25 ml of buffer - which is diluted in buffer extemporaneously.

Preparation of the plates.

The plates which are used have 96 U-shaped wells (NUNC= ELISA). They include 12 rows of 8 wells each, numbered from 1 to 12.

The distribution of antigens is as follows :

- 100 µl of the viral antigen, diluted in carbonate buffer at pH 9.6, are deposited in each of the wells of rows (marked 9)

1 - 2 - 5 - 6 - 9 - 10

- 100 µl of the control antigen, diluted in carbonate buffer at pH 9.6, are deposited in each of the wells of rows (marked 9)

3 - 4 - 7 - 8 - 11 - 12.

The dilution of the viral antigen is titrated at each viral production. Several dilutions of viral antigen are tested and compared to positive and negative known controls (at several dilutions) and to human anti-IgG labelled with peroxydase, the latter being also tested at several dilutions.

As a rule, the proteic concentration of the preparation is of 5 to 2.5 µg/ml.

The same proteic concentration is used for the control antigen.

The plates are closed with a plastic lid and are incubated overnight at 4°C.

Then they are put once in distilled water and centrifuged. The wells are then filled with 300 µl of non foetal calf serum at 20 % in PBS buffer.

The incubation lasts 2 hours at 37°C (covered plates).

The plates are washed 3 times in PBS buffer with TWEEN 20, 0.05 % (PBS-tw buffer) :

. first washing 300 µl

. second and third washing 200 µl/well.

The plates are carefully dried and sealed with an adhesive plastic film. They can be stored at -80°C.

ELISA reaction : antibody titer assay.

After defreezing, the plates are washed 3 times in PBS-TWEEN. They are carefully dried.

The positive and negative control sera as well as the tested sera are diluted first in the tube, with PBS-TWEEN containing 0.5 % bovine albumine.

The chosen dilution is 1/40.

- 100 µl of each serum are deposited in duplicate on the viral antigen and in duplicate on the control antigen.
- The same is carried out for the positive and negative diluted sera.
- 100 µl of PBS + TWEEN + bovine serum albumine are introduced in two wells Θ and in two wells Θ to form the conjugated controls.

The plates equiped with their lids are incubated for 1 h 30 at 37°C.

They are washed 4 times in PBS + TWEEN 0.05 %.

- 100 µl of human anti-IgG (labelled with peroxydase) at the chosen dilution are deposited in each well and incubated at 37°C.

The plates are again washed 5 times with the (PBS + TWEEN) buffer. They are carefully dried.

Revealing the enzymatic reaction is carried out by means of a orthophenylene-diamine substrate (0.05 % in citrate buffer pH 5.6 containing 0.03 % of H_2O_2).

100 µl of substrate are distributed in each well.

The plates are left in a dark room 20 minutes at the laboratory temperature.

Reading is carried out on a spectrophotometer (for microplates) at 492 nm.

Sera deemed as containing antibodies again the virus are those which give a ODD (optical density difference = optical density of viral antigen less optical density of control antigen) equal or higher to 0.30.

This technique enables a qualitative titration as well as a quantitative one. For this purpose, it is possible either to use several dilutions of the serum to be assayed, or to compare a dilution of the serum with a range of controls tested under the same conditions.

The table hereafter provides first results of serological investigations for LAV antibodies, carried out by using the above exemplified ELISA assay.

FIRST RESULTS OF SEROLOGICAL INVESTIGATIONS FOR LAV ANTIBODIES IN FRANCE

	Total examined	ELISA-LAV		ELISA-HTLV1** (Biotech)	
		positive	%positive	positive	%positive
Lymphadenopathy patients*	35	22	(63)	5***	(14)
Healthy homosexuals	40	7	(17)	1	(3)
Control population	54	1	(1,9)	0	(<2,6)

* 28 homosexuals
3 Haitians (1 woman)
4 toxicomans (2 women)

** The number of positive sera is probably overestimated in this test, since no control of unspecific binding could be done.

*** Out of the 5 LAS HTLV1 positive; 3 were born in Haiti, 1 had stayed for a long time in Haiti and 1 had made several travels to USA.
All of them had also antibodies against LAV.

The table shows clearly high prevalence of LAV antibodies in the homosexual patients with LAS, the very low incidence in the normal population and also a moderate spread of virus infection in still healthy homosexuals. In the latter group, all the positive individuals had a high number of partners (> 50 per year). The incidence of HTLV antibodies was very low in all three groups (determined by using a commercial

ELISA test (Biotech)). The groups of AIDS patients gave less interpretable results : approximatively 20 % had LAV antibodies, but some of the sera were taken at a very late stage of the disease, with a possible negativation of the humoral response.

It should further be mentionned that lymphocytes of all LAS patients do not produce detectable amounts of LAV-type virus. Particularly cells of lymph nodes from 6 more LAS patients were put in culture and tested for virus production, as described for patient 1. No virus release could be detected by RT activity. However, a p25 protein recognized by the serum of the first patient could be detected in cytoplasmic extracts of the T-cells labelled with ^{35}S-methionine in 3 other cases. This suggests partial expression of a similar virus in such cases. Moreover, all (6/6) of these patients had antibodies against LAV p25 proteins, indicating that they all had been infected with a similar or identical virus.

Interestingly, in lymphocytes of one of the patients (patient 2), there was a weak but definite immunoprecipitation of a band of similar size (p24-p25) with goat antiserum raised against HTLV1. Similarly, the patient's serum had antibodies against both HTLV and LAV, suggesting a double infection by either viruses. Such cases seem rather unfrequent.

The invention finally also relates to the biological reagents that can be formed by the LAV extracts containing the p25 protein or by the purified p25 protein, particularly for the production of antibodies directed against p25 in animals or of monoclonal antibodies. These antibodies are liable of forming useful tools in the further study of antigenic determinants of LAV viruses or LAV-related viruses.

It is acknowledged that the OKT designations which have been used with respect to the designation of some sub-sets of lymphocytes or related monoclonal antibodies by way ease of language, should in no way be opposed to the validity of any corresponding trademark, whether registered or not by its owner.

It should further be mentionned that the viral extracts, particularly viral lysates or enriched fractions can also be defined by reference to their immunological relationship or similitude with the extracts or enriched fractions containing a p25 protein as obtainable from the strain LAV1, IDAV1 or IDAV2. Thus any protein fraction which is capable of giving similar patterns of immunological reaction as do the protein extracts of LAV1, IDAV1 or IDAV2 with the same sera, must be considered as equivalent thereof and, accordingly, be deemed as encompassed by the scope of the claims which follow. A similar conclusion extends of course to the diagnostic means (process and kits) which may make use of such equivalent protein extracts.

The LAV1 virus has been deposited at the "Collection Nationale des Cultures de Micro-organismes" (C.N.C.M.) under n° I-232 on July 15, 1983 and IDAV1 and IDAV 2 viruses have been deposited at the C.N.C.M. on September 15, 1983 under n° I-240 and I-241, respectively. The invention encompasses as well the extracts of mutants or variants of the above deposited strains as long as they possess substantially the same immunological properties.

CLAIMS:

1 - A method for the in vitro diagnosis of LAS or AIDS, which comprises contacting a serum, or other biological medium obtained from the patient to be diagnosed with a retrovirus extract obtained from a T-lymphotropic-retrovirus, whose preferential target consists of Leu 3 cells, which has reverse transcriptase activity requiring the presence of Mg^{2+} ions and displaying a strong affinity for poly(adenylate-oligodeoxy-thymidylase) /poly(A)-oligo(dT) 12-18/, which has a density of 1.16 in a sucrose gradient, which has a mean diameter of 139 nanometre and a core of mean diameter of 41 nanometre, whose envelope proteins are not detected immunologically by the sera of LAS and AIDS afflicted patients whose lysates are recognized immunologically by said sera, said lysates containing a p25 protein which is recognised by the same sera but which is not recognized immunologically by p24 protein of HTLV virus, and detecting the immunological reaction.

2 - The method of claim 1 in which said retrovirus extract is obtainable from any of the retrovirus deposited at the C.M.C.M. under N° I-232, I-240 and I-241

3 - The method of claim 2 in which said retroviral extract is immunologically related to the retroviral extracts of claim 1 or 2.

4 - The method of any of claims 1 to 3 wherein said retroviral extract consists of the crude lysate of said retrovirus.

5 - The method of any one of claims 1 to 4 wherein said retrovirus extract is formed of the abovesaid purified p25 protein.

6 - The method of any one of claims 1 to 4

wherein the retrovirus extract consists of a purified protein having a molecular weight of approximately 12 000 (p12).

7 - The method of any one of claims 1 to 4 wherein the retrovirus extract consists of a purified protein having a molecular weight of approximately 18 000 (p18)

8 - The method of any one of claims 1 to 4 wherein said retrovirus extract is an enriched extract containing the abovesaid p25, p12 and p18 proteins.

9 - A kit for assay of sera from LAS or AIDS afflicted patients which comprises

- a retroviral extract as defined in any of claims 1 to 8, said retroviral extract being labelled;
- human anti-immunoglobulines;
- a lymphocyte extract obtained from a healthy person;
- buffers and, if appropriate, substrates for the vizualisation of the label;
- means to detect the labelled conjugate resulting from the immunological reaction between the labelled reagent and the assayed serum.

10 - A kit for AIDS or LAS assay which comprises

- a retroviral extract as defined in any of claims 1 to 8;
- labelled human anti-immunoglobulins;
- a lymphocyte extract obtained from a healthy person,
- buffers and, if appropriate, substrate for the vizualisation of the label;
- means to detect the labelled conjugate resulting from the immunological reaction between the labelled reagent and the assayed serum.

11. A method of claim 1 substantially as hereinbefore described.

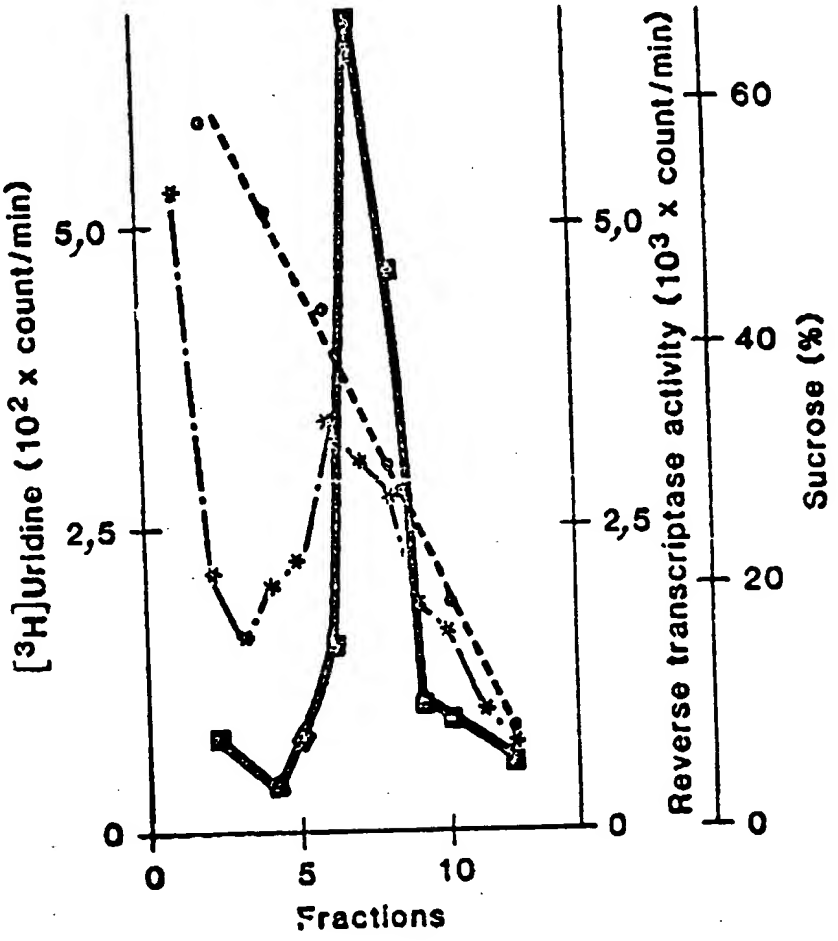
12. A kit for assay of sera from LAS or AIDS afflicted patients substantially as hereinbefore described.

13. A kit for AIDS or LAS assay substantially as hereinbefore described.

DATED THIS 6TH DAY OF SEPTEMBER 1984

SPOOR AND FISHER
PATENT ATTORNEYS FOR THE APPLICANT

INSTITUT PASTEUR, Etablissement Public
COMPLETE SPECIFICATION
APPLICATION NO. 84/7005



——— measure of reverse transcriptase activity on successive fractions of sucrose gradient.

—·—·— measure of acido precipitable material labelled with [^{3}H] uridine.

– – – – – density variation of the gradient.